

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
25 November 2004 (25.11.2004)

PCT

(10) International Publication Number
WO 2004/100980 A1

(51) International Patent Classification⁷: **A61K 38/21**,
31/7056, A61P 31/14

(21) International Application Number:
PCT/GB2004/002183

(22) International Filing Date: 19 May 2004 (19.05.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0311451.9 19 May 2003 (19.05.2003) GB
0320018.5 27 August 2003 (27.08.2003) GB
60/549,575 3 March 2004 (03.03.2004) US

(71) Applicant (for all designated States except US): **VIRAGEN, INC.** [US/US]; 865 S.W. 78th Avenue, Plantation, FL 33324 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **JERVIS, Karen, Elizabeth** [GB/GB]; Viragen (Scotland) Limited, Bush Loan, Pentlands Science Park, Penicuik, Midlothian EH26 0PZ (GB).

(74) Agent: **MURGITROYD & COMPANY**; Scotland House, 165-169 Scotland Street, Glasgow G5 8PL (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

Published:

— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: INTERFERON FOR TREATING OR PREVENTING A CORONAVIRAL INFECTION

(57) Abstract: The present invention provides a composition and method for use in the prevention or treatment of a coronaviral infection and in particular, the human coronavirus infection termed severe acute respiratory syndrome (SARS) coronavirus (SARS-HCoV). A method of treating a coronaviral infection is provided through the administration of interferon, further the use of interferons in the treatment of a coronaviral infection is also provided. Preferred forms of interferon for use in the invention are multi-subtype interferon products such as multi-subtype, human alpha-interferon derived from white blood cells commercially available as Multiferon.



WO 2004/100980 A1

BEST AVAILABLE COPY

1 INTERERON FOR TREATING OR PREVENTING A CORONAVIRAL INFECTION

2

3 **Field of the Invention**

4 The present invention provides a composition for use
5 in the treatment or prevention of a coronavirus
6 infection, more specifically a human coronaviral
7 infection, most specifically severe acute
8 respiratory syndrome (SARS) coronavirus.

9

10 **Background of the Invention**

11 Viral Infection

12 Viral infection is initiated by the binding of a
13 viral particle to a receptor on the surface of a
14 host cell membrane. The virus passes into the cell
15 by endocytosis. Enzymes encoded by the viral genome
16 are transcribed by the host cell and cause the viral
17 coat to fuse with the endosome membrane causing the
18 viral genome to be released into the cytosol. The
19 virus uses the host cell to effect protein
20 production in order to make numerous copies of the
21 genome. Viral coats are formed from coat proteins

1 encoded by the viral genome and synthesised by host
2 cell ribosomes. The viral genomes are then packaged
3 into the newly produced viral coats and expelled
4 from the host cell via the intracellular protein
5 trafficking pathway or through cell lysis. The
6 newly synthesised viral particles are then available
7 for infection of other host cells.

8

9 Coronaviruses

10 Members of the order *Nidovirales*, the coronaviruses
11 are enveloped, single stranded RNA viruses.
12 Coronaviral infection causes severe disease of the
13 respiratory and enteric systems. Coronaviruses have
14 been associated with gastroenteritis, hepatitis,
15 peritonitis and bronchitis. However, infection in
16 humans generally results in milder symptoms. The
17 SARS human coronavirus (SARS-HCoV) appears to be the
18 first coronavirus which regularly causes severe
19 disease in humans. SARS-HCoV causes severe
20 pneumonia-like symptoms in those infected, with
21 mortality occurring in the most severe cases.

22

23 Treatment of SARS

24 Various anti-viral treatments have been administered
25 to humans infected with SARS-HCoV, including general
26 anti-virals, treatments which inhibit viral cell
27 entry or replication, and immunostimulants.

28

29 Ribavirin is a broad spectrum anti-viral agent based
30 on a purine nucleoside analogue and is the standard
31 treatment regimen for hepatitis C. Ribavirin is
32 known to be active against various RNA viruses by

1 inducing lethal mutagenesis of the viral RNA genome
2 (Crotty *et al.*, 2000; Tam *et al.*, 2001) and is known
3 to show anti-viral activity against animal
4 coronaviruses (Weiss & Oostrom-Ram, 1989; Sidwell *et*
5 *al.*, 1987). However, *in vitro* tests of the efficacy
6 of the drug against SARS-HCoV have produced a series
7 of negative results and adverse reactions have also
8 been reported.

9
10 A limited number of other drugs have undergone
11 testing. The influenza drug, Oseltamivir, a
12 neuramidase inhibitor, has undergone analysis for
13 its efficacy against SARS-HCoV infection, but has
14 not shown any therapeutic benefit (Lee *et al.*, 2003
15 and Poutanen *et al.*, 2003). In laboratory tests,
16 Cystatin C, a protease inhibitor found in human
17 blood, was found to block replication of the 'common
18 cold' coronaviruses, but this has not been tested
19 against SARS-HCoV. It is unlikely that Cystatin C
20 will be a candidate for the treatment of SARS-HCoV
21 infected patients, since it has not undergone the
22 safety and efficacy tests required for all human
23 therapeutics.

24

25 Interferons

26 The interferons (IFNs) may be classified into two
27 distinct types - Type I IFNs and the Type II IFNs.
28 The type I IFNs consist of IFN alpha and IFN beta,
29 whereas the Type II group consists of IFN gamma.
30 Type I IFNs are produced in direct response to a
31 viral infection.

32

1 IFN alpha is represented by a large family of
2 structurally related genes expressing at least
3 thirteen subtypes, whereas IFN beta is encoded by a
4 single gene (Diaz et al., 1996). Both types of IFN
5 are able to stimulate an anti-viral state in target
6 cells, whereby the replication of a virus is
7 inhibited through the synthesis of enzymes which
8 interfere with the cellular and viral processes.

9
10 Type I IFNs also act to inhibit or slow the growth
11 of target cells and may render them more susceptible
12 to apoptosis. This has the effect of limiting the
13 extent of viral spread. Type I IFNs are
14 immunomodulators, or 'biological response modifiers'
15 which act to stimulate the immune response. Even
16 though IFN alpha and IFN beta show many broad
17 similarities in their actions, there are significant
18 differences in the manner by which they exert their
19 effects and it is these extended functions that
20 account for the different ranges of antiviral
21 activities of the two types. A review of the
22 different mechanisms by which interferons exert
23 their anti-viral effects is provided by Goodbourn et
24 al., 2000.

25
26 Recombinant interferons, which consist of only the
27 IFN alpha 2 subtype, currently dominate the market
28 for anti-viral and oncology indications. The two
29 main recombinant alpha IFN products, Intron ATM from
30 Schering Plough (IFN-alpha 2b) and RoferonTM (IFN-
31 alpha 2a) from Roche. In contrast to these single-
32 subtype products, there are several alpha IFN

1 preparations that consist of a mixture of different
2 subtypes. These multi-subtype IFN alpha products
3 are produced either by human leukocytes in response
4 to a stimulation from a virus (such as Multiferon™
5 from Viragen, Inc or its subsidiaries, or Alferon-N™
6 from Interferon Sciences/Hemispherix), or in human
7 lymphoblastoid cells, cultured from a patient with
8 Burkitt's lymphoma (such as Sumiferon™ from
9 Sumitomo).

10

11 There are many differences between the recombinant
12 forms of IFN alpha and the multi-subtype forms. The
13 most obvious difference is the number of IFN alpha
14 subtypes each possesses. As mentioned previously,
15 the recombinant forms comprise only the alpha 2
16 subtype - the alpha 2b form for Intron A™ (Schering
17 Plough) and the alpha 2a form for Roferon™ (Roche).
18 These two allelic variants differ by only one amino
19 acid residue. The multi-subtype forms of IFN alpha,
20 as the name suggests, comprise many subtypes of IFN
21 alpha. Another difference between the multi-subtype
22 and the recombinant forms is that the IFN alpha 2
23 produced by human cells in the manufacturing process
24 of the multi-subtype forms is glycosylated, whereas
25 the recombinant forms are unglycosylated, in that
26 they are produced through bacterial fermentation.
27 Glycosylation plays a major role in many functions
28 of the protein product, such as half-life, the
29 bioactivity and its immunogenicity. Therefore, the
30 glycosylation of a product is an important
31 consideration when developing a therapeutic or
32 prophylactic treatment, as it may affect the

1 duration in the body after administration, the
2 activity of a therapeutically appropriate dose and
3 the tolerability to the product itself.
4

5 During the last decade, considerable progress has
6 been achieved in the identification of the
7 components, as well as the molecular events involved
8 in the immunotherapeutic effects of interferons.
9 Over thirty different proteins have been identified
10 that have been shown to be induced by interferon
11 (Strannegard, 2002, unpublished review).
12

13 There are currently no completely effective
14 therapeutic or prophylactic treatments for humans
15 infected with coronavirus and in particular SARS-
16 HCoV. There thus exists a need for an effective
17 treatment for coronaviral infection in humans, and
18 in particular for severe acute respiratory syndrome
19 (SARS) coronavirus.
20

21 **Summary of the Invention**

22

23 The present inventors have now shown that
24 interferons and in particular multiple subtype
25 natural human alpha interferon products are
26 surprisingly effective at treating human coronavirus
27 infection, and in particular severe acute
28 respiratory syndrome (SARS) coronavirus.
29

30 According to a first aspect of the present invention
31 there is provided a method of treating coronaviral
32 infection, the method including the step of

1 administering a therapeutically useful amount of an
2 interferon to a subject in need of treatment.

3

4 In one preferred embodiment, the method of treatment
5 can be used to prevent coronaviral infection, the
6 method including the step of administering a
7 therapeutically useful amount of an interferon to a
8 subject sufficient to cause protection against
9 infection.

10

11 Interferon in each or any of the aspects of the
12 invention is preferably isolated interferon. An
13 isolated interferon is an interferon which is
14 synthetic (e.g. recombinant), or which is altered,
15 removed or purified from the natural state through
16 human intervention. For example, an interferon
17 naturally present in a living animal is not
18 isolated, whereas a synthetic interferon, or an
19 interferon which is partially or completely
20 separated from the coexisting materials of its
21 natural state, is isolated. An isolated interferon
22 can exist in substantially purified form, or can
23 exist in a non-native environment such as, for
24 example, a cell into which the interferon has been
25 introduced. Interferons purified from human cells,
26 for example the multi-subtype, human alpha-
27 interferon derived from white blood cells
28 commercially available as Multiferon™ from Viragen,
29 Inc. or any of its subsidiaries, are also considered
30 to be isolated molecules for purposes of the present
31 invention.

32

1 The interferon may be any suitable interferon, for
2 example interferon alpha or interferon beta. It may
3 be single or multi-subtype, but is preferably multi-
4 subtype.

5
6 The interferon may be naturally derived, for example
7 from human cells or recombinant, but preferably the
8 interferon is naturally derived. Preferably the
9 naturally derived interferon is obtained from
10 leukocytes following viral stimulation or produced
11 in human lymphoblastoid cells cultured from a
12 patient with Burkitt's lymphoma.

13
14 Preferred interferons for use in the invention
15 include multi-subtype interferon alpha (IFN α),
16 interferon α 1, interferon α 3 or interferon β 1b. A
17 particularly preferred interferon for use in the
18 invention is the multi-subtype IFN α product
19 commercially available from Viragen, Inc. or any of
20 its subsidiaries under the trade name MultiferonTM.

21
22 As used herein the term MultiferonTM refers to a
23 highly purified, multi-subtype, human alpha
24 interferon derived from human white blood cells
25 commercially available from Viragen, Inc or any of
26 its subsidiaries.

27
28 According to a second aspect of the present
29 invention there is provided an interferon for use in
30 the treatment or prevention of a coronaviral
31 infection.

32

1 Preferably the interferon is an isolated interferon.

2

3 The interferon may be any suitable interferon, for
4 example interferon alpha or interferon beta. It may
5 be single or multi-subtype, but is preferably multi-
6 subtype.

7

8 The interferon may be naturally derived, for example
9 from human cells or recombinant, but preferably the
10 interferon is naturally derived. Preferably the
11 naturally derived interferon is obtained from
12 leukocytes following viral stimulation or produced
13 in human lymphoblastoid cells cultured from a
14 patient with Burkitt's lymphoma.

15

16 Preferred interferons for use in the invention
17 include multi-subtype interferon alpha (IFN α),
18 interferon α 1, interferon α 3 or interferon β 1b. A
19 particularly preferred interferon for use in the
20 invention is the multi-subtype IFN α product
21 commercially available from Viragen, Inc. or any of
22 its subsidiaries under the trade name MultiferonTM.

23

24 As used herein the term MultiferonTM refers to a
25 highly purified, multi-subtype, human alpha
26 interferon derived from human white blood cells
27 commercially available from Viragen, Inc or any of
28 its subsidiaries.

29

30 According to a third aspect of the present invention
31 there is provided the use of an interferon in the

1 preparation of a medicament for the treatment or
2 prevention of a coronaviral infection.

3

4 Preferably the interferon is an isolated interferon.

5

6 The interferon may be any suitable interferon, for
7 example interferon alpha or interferon beta. It may
8 be single or multi-subtype, but is preferably multi-
9 subtype.

10

11 The interferon may be naturally derived, for example
12 from human cells or recombinant, but preferably the
13 interferon is naturally derived. Preferably the
14 naturally derived interferon is obtained from
15 leukocytes following viral stimulation or produced
16 in human lymphoblastoid cells cultured from a
17 patient with Burkitt's lymphoma.

18

19 Preferred interferons for use in the invention
20 include multi-subtype interferon alpha (IFN α),
21 interferon α 1, interferon α 3 or interferon β 1b. A
22 particularly preferred interferon for use in the
23 invention is the multi-subtype IFN α product
24 commercially available from Viragen, Inc. or any of
25 its subsidiaries under the trade name MultiferonTM.

26

27 As used herein the term MultiferonTM refers to a
28 highly purified, multi-subtype, human alpha
29 interferon derived from human white blood cells
30 commercially available from Viragen, Inc or any of
31 its subsidiaries.

32

1 Preferably the coronaviral infection is a human
2 coronaviral infection. Most preferably the
3 coronaviral infection is severe acute respiratory
4 system (SARS) coronavirus (SARS-HCoV).
5

6 According to a fourth aspect of the present
7 invention there is provided a method of treating or
8 preventing human infection with a coronavirus, and
9 in particular severe acute respiratory system (SARS)
10 coronavirus (SARS-HCoV), the method including the
11 step of administering a therapeutically useful
12 amount of an interferon to a subject in need of
13 treatment along with a therapeutically useful amount
14 of a suitable anti-viral compound.
15

16 In one preferred embodiment, the method of treatment
17 includes the prevention of human infection with a
18 coronavirus, wherein the method includes the step of
19 administering a therapeutically useful amount of an
20 interferon, or administering an amount of an
21 interferon along with an amount of a suitable anti-
22 viral compound sufficient to cause protection
23 against the infection.
24

25 Preferably the interferon is an isolated interferon.
26

27 Preferably the anti-viral compound is ribavirin.
28

29 Preferably the interferon is any suitable
30 interferon, for example interferon alpha or
31 interferon beta. It may be single or multi-subtype,
32 but is preferably multi-subtype.

1

2 The interferon may be naturally derived, for example
3 from human cells or of recombinant form, but
4 preferably the interferon is naturally derived.
5 Preferably the naturally derived interferon is
6 obtained from leukocytes following viral stimulation
7 or produced in human lymphoblastoid cells cultured
8 from a patient with Burkitt's lymphoma.

9

10 Preferred interferons for use in the invention
11 include multi-subtype interferon alpha ($\text{IFN}\alpha$),
12 interferon $\alpha 1$, interferon $\alpha 3$ or interferon $\beta 1b$. A
13 particularly preferred interferon for use in the
14 invention is the multi-subtype $\text{IFN}\alpha$ product
15 commercially available from Viragen, Inc. or any of
16 its subsidiaries under the trade name MultiferonTM.

17

18 As used herein the term MultiferonTM refers to a
19 highly purified, multi-subtype, human alpha
20 interferon derived from human white blood cells
21 commercially available from Viragen, Inc or any of
22 its subsidiaries.

23

24 According to a fifth aspect of the present invention
25 there is provided the use of interferon and an anti-
26 viral compound in the preparation of a combined
27 medicament for the treatment or prevention of
28 infection with a coronavirus, and in particular
29 severe acute respiratory system (SARS) coronavirus
30 (SARS-HCoV).

31

32 Preferably the interferon is an isolated interferon.

1

2 Preferably the anti-viral compound is ribavirin.

3

4 Preferably the interferon is any suitable
5 interferon, for example interferon alpha or
6 interferon beta. It may be single or multi-subtype,
7 but is preferably multi-subtype.

8

9 The interferon may be naturally derived, for example
10 from humans cell, or of recombinant form, but
11 preferably the interferon is naturally derived.

12 Preferably the naturally derived interferon is
13 obtained from leukocytes following viral stimulation
14 or produced in human lymphoblastoid cells cultured
15 from a patient with Burkitt's lymphoma.

16

17 Preferred interferons for use in the invention
18 include multi-subtype interferon alpha (IFN α),
19 interferon α 1, interferon α 3 or interferon β 1b. A
20 particularly preferred interferon for use in the
21 invention is the multi-subtype IFN α product
22 commercially available from Viragen, Inc. or any of
23 its subsidiaries under the trade name MultiferonTM.

24

25 As used herein the term MultiferonTM refers to a
26 highly purified, multi-subtype, human alpha
27 interferon derived from human white blood cells
28 commercially available from Viragen, Inc or any of
29 its subsidiaries.

30

31 The term 'treatment' as used herein refers to any
32 regime that can benefit a human or non-human animal.

1 The treatment may be in respect of an existing
2 condition or may be prophylactic (preventative
3 treatment). Treatment may include curative,
4 alleviation or prophylactic effects.

5

6 Administration

7 Interferons of and for use in the present invention
8 may be administered alone, or in combination with
9 another agent, but will preferably be administered
10 as a pharmaceutical composition, which will
11 generally comprise a suitable pharmaceutical
12 excipient, diluent or carrier selected dependent on
13 the intended route of administration.

14

15 Interferons of and for use in the present invention
16 may be administered to a patient in need of
17 treatment via any suitable route. The precise dose
18 will depend upon a number of factors, including the
19 precise nature of the interferon.

20

21 Some suitable routes of administration include (but
22 are not limited to) oral, rectal, nasal, topical
23 (including buccal and sublingual), vaginal or
24 parenteral (including subcutaneous, intramuscular,
25 intravenous, intradermal, intrathecal and epidural)
26 administration, or administration via oral or nasal
27 inhalation.

28

29 In preferred embodiments, the composition is
30 deliverable as an injectable composition, is
31 administered orally, is administered to the lungs as
32 an aerosol via oral or nasal inhalation.

1

2 For administration via the oral or nasal inhalation
3 routes, preferably the active ingredient will be in
4 a suitable pharmaceutical formulation and may be
5 delivered using a mechanical form including, but not
6 restricted to an inhaler or nebuliser device.

7

8 Further, where the oral or nasal inhalation routes
9 are used, administration by a SPAG (small
10 particulate aerosol generator) may be used.

11

12 For intravenous injection, the active ingredient
13 will be in the form of a parenterally acceptable
14 aqueous solution which is pyrogen-free and has
15 suitable pH, isotonicity and stability. Those of
16 relevant skill in the art are well able to prepare
17 suitable solutions using, for example, isotonic
18 vehicles such as Sodium Chloride Injection, Ringer's
19 Injection, Lactated Ringer's Injection.
20 Preservatives, stabilisers, buffers, antioxidants
21 and/or other additives may be included, as required.

22

23 Pharmaceutical compositions for oral administration
24 may be in tablet, capsule, powder or liquid form. A
25 tablet may comprise a solid carrier such as gelatin
26 or an adjuvant. Liquid pharmaceutical compositions
27 generally comprise a liquid carrier such as water,
28 petroleum, animal or vegetable oils, mineral oil or
29 synthetic oil. Physiological saline solution,
30 dextrose or other saccharide solution or glycols
31 such as ethylene glycol, propylene glycol or
32 polyethylene glycol may be included.

1
2 The composition may also be administered via
3 microspheres, liposomes, other microparticulate
4 delivery systems or sustained release formulations
5 placed in certain tissues including blood. Suitable
6 examples of sustained release carriers include
7 semipermeable polymer matrices in the form of shared
8 articles, e.g. suppositories or microcapsules.
9 Implantable or microcapsular sustained release
10 matrices include polylactides (US Patent No. 3,773,
11 919 and European Patent Application Publication No
12 0,058,481) copolymers of L-glutamic acid and gamma
13 ethyl-L-glutamate (Sidman *et al.*, Biopolymers 22(1):
14 547-556, 1985), poly (2-hydroxyethyl-methacrylate)
15 or ethylene vinyl acetate (Langer *et al.*, J. Biomed.
16 Mater. Res. 15: 167-277, 1981, and Langer, Chem.
17 Tech. 12:98-105, 1982, the entire disclosures of
18 which are herein incorporated by reference).

19
20 Examples of the techniques and protocols mentioned
21 above and other techniques and protocols which may
22 be used in accordance with the invention can be
23 found in Remington's Pharmaceutical Sciences, 16th
24 edition, Oslo, A. (ed), 1980, the entire disclosure
25 of which is herein incorporated by reference

26

27 Pharmaceutical Compositions

28 As described above, the present invention extends to
29 a pharmaceutical composition for the treatment or
30 prevention of a coronaviral infection, wherein the
31 composition comprises at least one interferon.
32 Pharmaceutical compositions according to the present

1 invention, and for use in accordance with the
2 present invention may comprise, in addition to
3 active ingredient (i.e. one or more interferons), a
4 pharmaceutically acceptable excipient, carrier,
5 buffer stabiliser or other materials well known to
6 those skilled in the art. Such materials should be
7 non-toxic and should not interfere with the efficacy
8 of the active ingredient. The precise nature of the
9 carrier or other material will depend on the route
10 of administration, which may be, for example, oral,
11 intravenous, or intranasal.

12

13 The formulation may be a liquid, for example, a
14 physiologic salt solution containing non-phosphate
15 buffer at pH 6.8 to 7.6, or a lyophilised powder.

16

17 Dose

18 The composition/interferon is preferably
19 administered to an individual in a "therapeutically
20 effective amount", this being sufficient to show
21 benefit to the individual. The actual amount
22 administered, and rate and time-course of
23 administration, will depend on the nature and
24 severity of what is being treated. Prescription of
25 treatment, e.g. decisions on dosage etc, is
26 ultimately within the responsibility and at the
27 discretion of general practitioners and other
28 medical doctors, and typically takes account of the
29 disorder to be treated, the condition of the
30 individual patient, the site of delivery, the method
31 of administration and other factors known to
32 practitioners.

1
2 The optimal dose can be determined by physicians
3 based on a number of parameters including, for
4 example, age, sex, weight, severity of the condition
5 being treated, the active ingredient being
6 administered and the route of administration.

7
8 For example, in one embodiment, a suitable dose of
9 interferon may be 1 to 10 million IU, for example 3
10 to 5 million IU three times weekly to 0.5 to 10
11 million, for example 2 to 8 million, or 4 to 6
12 million IU daily, although other doses may be used.

13
14 According to a further aspect of the present
15 invention there is provided an assay method for
16 determining the efficacy of a composition in the
17 treatment or prevention of a coronaviral infection,
18 wherein the composition comprises an interferon,
19 preferably a multi sub-type interferon.

20
21 In a further aspect of the present invention, there
22 is provided an assay method for determining the
23 efficacy of a candidate agent in the treatment of a
24 coronaviral infection, wherein the assay method
25 includes the steps of;

- 26 - incubating virus infected cells in the
27 presence of the candidate agent, and
28 - determining the degree of inhibition of the
29 cytopathic effect of the virus on the cells.

30

31

1 Preferably the method includes the further step of
2 comparing the degree of viral inhibition obtained
3 using the candidate agent with the degree of viral
4 inhibition obtainable with incubation with an
5 interferon or interferon based product.

6

7 Preferably the interferon is a multi-subtype
8 interferon, most preferably MultiferonTM.

9

10 In a still further aspect, there is provided an
11 assay method for determining the efficacy of a
12 candidate agent in the prevention of a coronaviral
13 infection, wherein the assay method includes the
14 steps of:

15 -incubating cells in the presence of the candidate
16 agent,

17 -adding the coronavirus to the cells, and

18 -determining the degree of protection against the
19 coronaviral infection afforded by the candidate
20 agent

21

22 Preferred assays for use in the assay methods of the
23 invention include cytopathic endpoint assays and
24 plaque reduction assays.

25

26 Preferred features of each aspect of the invention
27 are as for each of the other aspects *mutatis*
28 *mutandis* unless the context demands otherwise.

29

30 Unless otherwise defined, all technical and
31 scientific terms used herein have the meaning

1 commonly understood by a person who is skilled in
2 the art in the field of the present invention.

3
4 Throughout the specification, unless the context
5 demands otherwise, the terms 'comprise' or
6 'include', or variations such as 'comprises' or
7 'comprising', 'includes' or 'including' will be
8 understood to imply the inclusion of a stated
9 integer or group of integers, but not the exclusion
10 of any other integer or group of integers.

11

12 **Detailed description of the Invention**

13

14 The present invention will now be described with
15 reference to the following examples which are
16 provided for the purpose of illustration and are not
17 intended to be construed as being limiting on the
18 present invention, and further, with reference to
19 the figures.

20

21 **Brief description of the drawings**

22

23 Figure 1 shows a dose response curve produced
24 from an *in vitro* plaque reduction assay,
25 showing that with increasing concentrations of
26 the MultiferonTM, the effect of the SARS-HCoV
27 virus is attenuated;

28

29 Figure 2 shows the effect of MultiferonTM and
30 Intron ATM on the cytopathogenicity of Semliki
31 Forest Virus (SFV) on African Green Monkey
32 Kidney Vero E6 cells;

Figure 3 shows the effect of Multiferon™ on the cytopathogenicity of human Encephalomyocarditis virus (EMCV) on human A459 cells, wherein the Multiferon™ concentration required to obtain 50% cytopathic effect (CPE) for human A459 cells challenged with EMC virus is shown for different concentrations of EMC virus, presented as a 1/dilution; and

Figure 4 shows the effect of increasing concentrations of Multiferon™ on human A459 cell survival. Cell survival was measured photometrically at Abs_{595nm} using a fixed dilution of EMC virus (dilution 1/400), at increasing concentrations of Multiferon. AU denoted Absorbance Units.

Examples

Example 1 - Anti-viral effect of interferon against SARS-HCoV infection in Vero E6 cells

The effectiveness of the interferons to inhibit the cytopathic effect following SARS-HCoV infection was tested in a cytopathic endpoint assay and a plaque reduction assay. All endpoint assays were carried out using the multi-subtype interferons Multiferon™ and interferon α 3, as well as single subtype recombinant interferon alpha (subtypes interferon α 2a, interferon α 2b, and interferon α 1) and the interferon beta (IFN β) subtypes interferon β 1a and

1 interferon β 1b as well as the anti-viral Ribavirin
2 for comparison.

3

4 Preparation of anti-viral treatments

5 A broad range of concentrations (obtained by ten-
6 fold dilutions) encompassing the inhibitory dosages
7 stated by the manufacturer for other viral-host
8 combinations was tested. Compounds already present
9 in aqueous injections were made up to volume using
10 Hank's buffered saline solution. For tablet and
11 capsule formulations with soluble active
12 ingredients, the outer coat was removed wherever
13 applicable and the preparation ground in a mortar
14 and pestle. The contents were dissolved in water,
15 vortexed and centrifuged thereafter at 3000G. The
16 required volume was pipetted from the supernatant
17 and diluted accordingly. Where active ingredients
18 were insoluble in water, the contents were dissolved
19 in dimethylsulphoxide (DMSO) and care was taken to
20 ensure that the final concentration of DMSO in the
21 dilutions would not exceed 1%. For plaque assays,
22 5-fold drug dilutions were prepared using growth
23 media as specified below.

24

25 SARS-HCoV production and infection

26 African Green Monkey (Vero E6) cells (American Type
27 Culture Collection, Manassas, VA, USA) were
28 propagated in 75cm² cell culture flasks containing
29 growth medium consisting of medium 199 (Sigma, St
30 Louis, USA) supplemented with 10% foetal calf serum
31 (FCS; Biological Industries, Israel). SARS-HCoV
32 2003VA2774 (an isolate from a SARS patient in

1 Singapore) was propagated in Vero E6 cells.
2 Briefly, 2 ml of stock virus was added to a
3 confluent monolayer of Vero E6 cells and incubated
4 at 37°C in 5% CO₂ for one hour. 13 ml of medium 199
5 supplemented with 5% FCS was then added. The
6 cultures were incubated at 37°C in 5% CO₂ and the
7 inhibition of cytopathic effect gauged by observing
8 each well through an inverted microscope. Where 75%
9 or greater inhibition was observed after 48 hours,
10 the supernatant was harvested. The supernatant was
11 clarified at 2500 rpm and then aliquoted into
12 cryovials and stored at -80°C until use.

13

14 Virus handling and titration

15 Virus titre in the frozen culture supernatant was
16 determined using a plaque assay carried out in
17 duplicate. Briefly, 100 microlitres of virus in 10-
18 fold serial dilution was added to a monolayer of
19 Vero E6 cells in a 24 well-plate. After incubation
20 for an hour at 37°C in 5% CO₂, the viral inoculum was
21 aspirated and 1 ml of carboxymethylcellulose overlay
22 with medium 199 supplemented with 5% FCS was added
23 to each well. After four days of incubation, the
24 cells were fixed with 10% formalin and stained with
25 2% crystal violet. The plaques were counted
26 visually and the virus titre in plaque forming units
27 per ml (pfu/ml) calculated.

28

29 Cytopathic endpoint assay

30 The protocol used was adapted from Al-Jabri *et al.*
31 1996. The effect of each anti-viral treatment was
32 tested in quadruplicate. Briefly, 100 microlitres

1 of serial 10-fold dilutions of each treatment were
2 incubated with 100 microlitres of Vero E6 cells
3 giving a final cell count of 20,000 cells per well
4 in a 96-well plate. Incubation was at 37°C in 5% CO₂
5 overnight for the interferon preparations and for
6 one hour for Ribavirin. 10 microlitres of virus at
7 a concentration of 10,000 pfu/well were then added
8 to each test well. This equates to a multiplicity
9 of infection (MOI) (virus particles per cell) of 0.5.
10 The plates were incubated at 37°C in 5% CO₂ for three
11 days and the plates were observed daily for
12 cytopathic effects. The end point was the diluted
13 concentration that inhibited the cytopathic effect
14 in all four set-ups (CIA₁₀₀).

15

16 To determine cytotoxicity, 100 microlitres of serial
17 10-fold dilutions of each treatment were incubated
18 with 100 microlitres of Vero E6 cells giving a final
19 cell count of 20,000 cells per well in a 96-well
20 plate, without viral challenge. The plates were
21 then incubated at 37°C in 5% CO₂ for three days and
22 toxicity effects were observed for using an inverted
23 microscope.

24

25 Interferons which showed complete inhibition were
26 tested further at the lower viral titres of 10³ and
27 10² pfu/well.

28

29 Plaque reduction assay

30 Multiferon™, interferon α3 and interferon β1b were
31 further tested using a plaque reduction assay.
32 Trypsinised Vero E6 cells were re-suspended in

1 growth medium and pre-incubated for 15 hours with a
2 serial 5-fold dilution of interferon α 3, interferon
3 β 1a and MultiferonTM in 24-well plates. The following
4 day, the medium was aspirated and 100 microlitres of
5 virus was added to each well at a titre of 100
6 pfu/well.

7
8 After incubation for one hour, the virus inoculum
9 was aspirated and a carboxymethylcellulose overlay
10 containing maintenance medium and the appropriate
11 interferon concentration was added. After four days
12 incubation, the plates were fixed and stained as
13 described above.

14
15 Viral plaques were visible 3 days after pre-
16 incubation of infected cells for 15 hours with five-
17 fold dilutions of the interferon. Plaques were then
18 counted visually and the concentration of the
19 interferon which inhibited 50% of plaques in each
20 well (IC₅₀) determined. Results were plotted in
21 Microsoft Excel, and a polynomial of order three was
22 used to approximate the data and extrapolate IC₅₀ and
23 IC₉₅ values. (Results not shown)

24
25 The assay was also carried out in duplicate as
26 described above for MultiferonTM at a viral titre of
27 54 pfu/well.

28
29 Interferons are known to be relatively species
30 specific as the target for the interferon is the
31 infected cell rather than the virus itself. The
32 anti-viral activity of MultiferonTM was also assessed

1 in a human cell line, the pulmonary epithelial cell
2 line A549.

3

4 Results

5

6 Cytopathic Endpoint Assay

7 The cytopathic effect of SARS-HCoV was evident
8 within 24 hours following infection. Infected cells
9 were rounded and exhibited monolayer destruction.

10

11 Complete inhibition using a high viral challenge
12 (10^4 pfu/well) and high multiplicity of infection
13 (0.5) was observed for RibavirinTM, and for the
14 MultiferonTM product. At a viral load of 10^2
15 pfu/well the CIA₁₀₀ value was 5 IU/ml for
16 MultiferonTM, with no cytotoxicity observed.

17

18 Although RibavirinTM showed inhibitory activity at
19 all viral titres this was only at high
20 concentrations of the drug. Such concentrations
21 showed cytotoxicity and thus RibavirinTM is not
22 likely to be a clinically effective treatment for
23 severe acute respiratory syndrome (SARS)
24 coronavirus.

25

26 In contrast, MultiferonTM did not show any
27 cytotoxicity at this inhibitory concentration.

28

29 Interferon α 3, interferon α 1 and interferon β 1b
30 also showed inhibition of cytopathic effect using
31 this assay. Interferon α 2a, interferon α 2b and

1 interferon β 1a did not show significant inhibition
2 (results not shown).

3

4 Results are shown for MultiferonTM and RibavirinTM in
5 Tables 1 and 2 below.

6

Anti-viral Treatment	Concentration at which complete cytopathic effect	CIA ₁₀₀
Multiferon TM	5,000 IU/ml	Yes
Ribavirin TM	5,000 μ g/ml	Yes

7 **Table 1:** Results of the Cytopathic Endpoint Assay
8 for MultiferonTM and RibavirinTM. (Results not shown
9 for other treatments tested)

10

Virus Load (pfu/well)	Multiferon TM (IU/ml)	Ribavirin TM (μ g/ml)
1,000	50	5,000
100	5	500

11 **Table 2:** Data obtained for MultiferonTM and the anti-
12 viral product, RibavirinTM. (Results not shown for
13 the other treatments tested).

14

15 Plaque Reduction Assay

16 The MultiferonTM preparation displayed a dose-
17 dependent inhibition of SARS-HCoV plaque formation.
18 IC₅₀ and IC₉₅ values for MultiferonTM treatment were 2
19 IU/ml and 44 IU/ml, respectively. Results are shown
20 below for MultiferonTM in Table 3 and in Figure 1 for
21 a viral titre of 54 pfu/well. An EC₅₀ value of 3.16
22 IU/ml was obtained.

1

Multiferon™ Concentration (IU/ml)	Log Multiferon™ Concentration (Log IU/ml)	% plaque reduction (Well 1)	% plaque reduction (Well 2)	Average plaque reduction
5000	3.69897	100	100	100
1000	3	100	100	100
200	2.30103	100	100	100
40	1.60206	100	100	100
8	0.90309	68.5	75.9	72.2
1.6	0.20412	40.7	48.1	44.4
0.32	-0.49485	18.5	25.9	22.2
0.064	-1.19382	0	0	0

2 **Table 3:** Results obtained in the plaque reduction
3 assay for Multiferon™ at 54 pfu/well.

4

5 Interferon α 3 and interferon β 1a also showed dose-
6 dependent inhibition of SARS-HCoV plaque formation
7 in this assay (results not shown).

8

9 Example 2

10

11 SARS-HCoV, strain Frankfurt-1, kindly provided by
12 the Bernard Notch Institute, Frankfurt, Germany, was
13 propagated on Vero E6 cells, an African Green Monkey
14 cell line obtained from American Type Culture
15 Collection, Manassas, VA, USA. For titration of the
16 virus, serial dilution of SARS-HCoV were added to
17 Vero E6 cells grown in micro-plates with Eagle's
18 medium containing 2% foetal calf serum. After 3
19 days of culture, cytopathogenic effects were
20 determined microscopically and cytotoxicity was then
21 assayed using a colorimetric assay based on the
22 measurement of lactate dehydrogenase (LDH) activity

1 released from the cytosol of damaged cells
2 (Cytotoxicity detection kit, Roche Diagnostics GmbH,
3 Penzberg, Germany).
4

5 For the antiviral experiments the following four
6 different commercially available interferon
7 preparations were used: 1) Intron ATM, Schering
8 Plough, USA; 2) RoferonTM, Roche, Switzerland; 3)
9 BetaferonTM, Schering AG, Germany and 4) MultiferonTM
10 (Viragen, Florida, USA).
11

12 Serial 5-fold dilutions (0.2-31.125 IU/ml) of the
13 interferon preparations were added to Vero E6 cells
14 in micro-plates which were then incubated overnight
15 at 37°C. SARS-HCoV was then added at different
16 concentrations (1000, 100 or 10 TCID₅₀) to different
17 sets of interferon dilutions, and after a further
18 incubation of 3 days the plates were read
19 microscopically, and then by the ELISA LDH
20 cytotoxicity assay.
21

22 In a separate set of experiments, the method used by
23 Cinatl et al. (2003) including addition of
24 interferon on two occasions, one day before and one
25 day after addition of the virus to the plates, was
26 employed.
27

28 In all experiments, controls with 1) virus but not
29 interferon, 2) all different dilutions of the
30 interferons but no virus, and 3) no virus and no
31 interferon were included.
32

1 Results

2 The cytotoxicity (LDH) assay used for determination
 3 of SARS-HCoV cytopathogenic effect (CPE) was found
 4 to be highly reliable, giving OD values in CPE-
 5 positive cultures of 1.5-1.8 and in CPE-negative
 6 cultures values not exceeding 0.2.

7
 8 Although two of the interferons, Roferon ATM and
 9 MultiferonTM showed a tendency to increase baseline
 10 levels in the cytotoxicity assay, the result showed
 11 no dose-dependent increase in these levels and the
 12 OD values did not exceed 0.6 in any case. There was
 13 no similar tendency for Intron ATM or BetaferonTM.
 14 The concentration of interferons capable of
 15 decreasing OD values of virus-infected cultures by
 16 50% (IC₅₀) are shown in Table 4 which shows the
 17 results of experiments where IFN was added either
 18 once (type 1) or twice (type 2) to the cells.

19

	IC ₅₀ Exp. Type 1		IL ₅₀ Exp. Type 2	
Interferon	10 TCID ₅₀	100 TCID ₅₀	10 TCID ₅₀	100 TCID ₅₀
Betaferon	110	625	110	190
Multiferon	540	2400	490	2200
Intron A	>3.125	>3.125	>3.125	>3.125
Roferon	>3.125	>3.125	>3.125	>3.125

20

21 **Table 4.** Effect of various interferons on SARS-HCoV
 22 replication

23

24 IC₅₀ values given as IU of interferon per ml. Slight
 25 inhibition of cytotoxicity was obtained with

1 Roferon™ as well as Intron A™ at the highest
2 concentrations tested, but the reduction of OD
3 values did not reach the 50% level in any experiment
4 with these interferons.

5
6 The outcome of the two different experiments
7 performed were similar, showing that Betaferon™ had
8 the highest antiviral activity (IC₅₀ 50-500 IU/ml)
9 followed by Multiferon™ (IC₅₀ 500-2000 IU/ml).
10 Neither Intron A™ nor Roferon™ had any clear
11 antiviral activity at the highest concentrations
12 used in the experiments (3.125 IU/ml). Extrapolation
13 of results obtained with the highest concentrations
14 of the IFN preparations showed that IC₅₀ levels could
15 be expected to be reached at concentrations of
16 10,000-15,000 IU/ml for the latter two types of IFN-
17 α.

18

19 Discussion

20 The present results corroborate earlier findings
21 that IFN-β has an antiviral activity against the
22 SARS-HCoV, that is superior to that of recombinant,
23 IFN-α2, interferons (Cinatl et al., 2003).
24 Furthermore, the results indicate that multi-
25 subtype, natural IFN-α, albeit being less active
26 that β-interferon, also has a significant effect on
27 SARS-HCoV replication. The latter finding agrees
28 with the recent results by Tan et al. (2004) who
29 found, using a plaque reduction assay, that two
30 types of natural IFN-α preparations showed strong

1 anti SARS-HCoV activity with a potency that was only
2 slightly lower than that obtained with β -interferon.

3
4 The accumulated evidence now suggests that
5 interferons may have a role in the treatment of
6 severe acute respiratory syndrome (SARS)
7 coronavirus. The promising results of Loutfy et al.
8 (2003) were obtained using a recombinant so-called
9 consensus IFN- α (Infergen) that is believed to have
10 effects that are shared by various subtypes of IFN-
11 α . The suggestive clinically beneficial effect of
12 the consensus IFN- α may be concordant with the
13 presently obtained in vitro results with nIFN- α , but
14 as far as we are aware, no studies on the relative
15 in vitro activities of nIFN- α and consensus IFN- α
16 have been performed.

17
18 Example 3 - Anti-viral effect of multi-subtype
19 interferon as compared to Intron A against Semliki
20 Forest Virus in Vero E6 cells

21
22 Vero E6 cells were seeded in 96-well plate, at a
23 density of 10000 cells per well. After incubation
24 overnight at 37°C, cells were incubated with 100ul
25 of a serial 10-fold dilution of Multiferon or Intron
26 A (titration range from 1250 IU/ml - 2.4 IU/ml).
27 After 24 hours, cells were infected with 5000 pfu of
28 Semliki Forest Virus (estimated MOI was 0.1) and
29 further incubated for 48 hours until cytopathic
30 effect was observed in untreated wells. Media was
31 removed from cells, and cells were washed in 1 x

1 PBS, then fixed for 10 minutes at room temperature
2 in 4% paraformaldehyde in PBS. Paraformaldehyde was
3 removed and cells were stained with 0.2% crystal
4 violet in 2% ethanol for 10 minutes at room
5 temperature. Stained plates were washed and degree
6 of colouration was quantified at 630nm using an
7 ELISA reader. Triplicate data is presented in graph
8 format (Figure 2).

9

10 Results

11 Figure 2 demonstrates that Multiferon was found to
12 be effective at protecting Vero E6 cells from SFV
13 infection over a range of concentrations. At 625
14 IU/ml, the same degree of protection was observed
15 for both Multiferon and IntronA (results not shown),
16 and an equivalent loss of protection was observed
17 for both products at 39 IU/ml. At all
18 concentrations in between, Multiferon provided
19 significantly higher protection than provided by
20 Intron A.

21

22 Example 4 - Anti-viral effect of multi-subtype 23 interferon in Human Cells

24

25 MultiferonTM was added prior to addition of the
26 virus. The human Encephalomyocarditis virus (EMCV)
27 was then used to infect A549 cells and the effect of
28 MultiferonTM on the cytopathogenicity of EMCV was
29 determined by assessing the interferon concentration
30 required to obtain 50% cytopathic effect (CPE) for
31 the human A549 cells. Results are shown in Figure

1 3. Cell survival was measured photometrically and
2 results are shown in Figure 4.

3
4 The results show that the Multiferon™ preparation
5 successfully protected against a cytopathic effect
6 on EMCV-infected cells and that the adverse effect
7 on the host cells did not continue to rise
8 significantly at effective Multiferon™
9 concentrations.

10
11 Figure 3 shows the concentrations of Multiferon™
12 needed to obtain 50% cytopathic effect in the human
13 cells at varying viral titres. As would be
14 expected, a higher viral concentration requires a
15 higher effective Multiferon™ concentration.

16
17 Figure 4 shows that Multiferon™ does not have
18 significant adverse cell toxicity effects on human
19 host cells.

20

21 Discussion

22 The results provided show that many interferons are
23 highly effective at inhibiting the activity of the
24 SARS-HCoV. Further, it has been shown that, in
25 general natural interferons, especially multi sub-
26 type interferons, such as Multiferon™, are
27 particularly effective. Moreover at effective
28 Multiferon™ concentrations, no cytotoxicity is
29 observed.

30

31 In tests for anti-viral activity in human cells,
32 Multiferon™ shows a good dose response with

1 cytotoxicity levels which do not rise in proportion
2 to the effective Multiferon™ concentration.

3

4 These results indicate that certain interferons such
5 as Multiferon™ are highly effective therapeutics for
6 the treatment of SARS-HCoV infection in humans and
7 can be expected to have low levels of adverse
8 effects *in vivo*.

9

10 Other groups have studied the efficacy of
11 recombinant interferon products against SARS CoV.
12 Stohr et al demonstrated significant but incomplete
13 activity of Intron A at a concentration of 1000-5000
14 IU/ml on cells infected with a multiplicity of
15 infection (MOI) of 0.001 plaque forming units per
16 cell in a cytopathic endpoint assay. However, the
17 results presented show that Multiferon™ used at the
18 low dose of 5 IU/ml completely protected cells from
19 SARS-HCoV infection at a MOI of 0.005 plaque forming
20 units per cell, five times greater than the MOI used
21 in the Intron A™ experiments. Furthermore, 50 IU/ml
22 of Multiferon™ protected cells from SARS-HCoV
23 infection at a MOI of 0.05, 50 times greater than
24 the MOI utilised in the Intron A™ studies. Finally,
25 in our studies, concentrations of Intron A™ or
26 Roferon™ up to 100000 and 500000 IU/ml,
27 respectively, failed to fully protect cells from
28 SARS-HCoV infection.

29

30 Whilst Stohr et al. claim that doses of up to $3.6 \times$
31 10^7 IU/ml have been infused intravenously, and that
32 serum concentrations of at least 500 IU/ml are

1 achievable after intramuscular injection, the serum
2 titre would only reach this level for a short period
3 of time, and intravenous infusion has highly toxic
4 implications. Taken together with the results
5 described, this supports the significant superiority
6 of natural multi-subtype interferon products, in
7 particular MultiferonTM, over recombinant IFN alpha2
8 preparations.

9
10 All publications and patent documents referred to
11 herein are incorporated by reference in their
12 entirety. Although the invention has been described
13 in connection with specific examples, it should be
14 understood that the invention should not be unduly
15 limited to such examples. Specifically, it will be
16 understood by one skilled in the art that various
17 modifications to and variations of the invention as
18 described herein may be made without departing from
19 the scope of the invention.

20

21 **References**

22

23 Al-Jabri *et al.* In Mahy, BWJ and Kangro, HO eds.
24 *Virology Methods Manual*, Academic Press Ltd, London
25 (1996). 293-356

26

27 Cinatl, J *et al.* *Lancet* **362** (9380) 293-294

28

29 Crotty, S. *et al.* *Nat. Med* **6** 1375-1379

30

31 Goodbourn, S.E.Y., *et al.* (2000). *J. Gen. Virol.* **81**
32 2341-2364.

- 1
2 Lee, N. *et al.* *N. Engl. J. Med.* **348**(20) 1986-1994
3
4 Loutfy, M.R. *et al.*, *JAMA* **290**(24) 3251-3253
5
6 Poutanen, S.M. *et al.* (2003) *New Engl. J. Med.* **348**
7 **(20)** 1995-2005
8
9 Sidwell, R.W. *et al.* *Antimicrob. Agents Chemother.*
10 **31** 1130-1134
11
12 Stoher, U. *et al.* (2004). *Journal of Infectious*
13 *Diseases.* 189:1164-7
14
15 Tam, R.C. *et al.* *Antivir.Chem. Chemother.* **12** (5)
16 261-272
17
18 Tan, E.L.C. *et al.* (2004). *Emerging infectious*
19 *diseases.* **10**(4) 581-586
20
21 Weck, P.K. *et al.* 1981. *J. Gen. Virol.* **57** 233-237
22
23 Weiss, R.C. & Oostrom-Ram, T. *Vet Microbiol.* **20** 255-
24 265

1 **Claims**

2

3 1. A method of treating or preventing a
4 coronaviral infection, the method including the
5 step of administering a therapeutically useful
6 amount of an interferon to a subject in need of
7 treatment.

8

9 2. A method as claimed in claim 1, wherein the
10 interferon is interferon alpha or interferon
11 beta.

12

13 3. A method as claimed in claim 1 or claim 2
14 wherein the interferon is selected from the
15 group consisting of multi-subtype interferon
16 alpha (IFN α), interferon α 1, interferon α 3 or
17 interferon β 1b.

18

19 4. A method as claimed in any one of claims 1 to 3
20 wherein the interferon is derived from human
21 cells.

22

23 5. A method as claimed in any one of claims 1 to 3
24 wherein the interferon is recombinant.

25

26 6. A method as claimed in any one of claims 1 to
27 5 wherein the interferon is an isolated
28 interferon.

29

30 7. A method as claimed in any preceding claim
31 wherein the interferon is multi-subtype, human

- 1 alpha-interferon derived from white blood cells
2 commercially available as Multiferon™.
3
- 4 8. A method as claimed in any preceding claim
5 wherein the coronavirus infection is a human
6 coronaviral infection.
7
- 8 9. A method as claimed in any preceding claim
9 wherein the coronaviral infection is severe
10 acute respiratory syndrome (SARS) coronavirus
11 (SARS-HCoV).
12
- 13 10. Use of interferon in the treatment of a
14 human coronaviral infection.
15
- 16 11. Use of interferon in the prevention of a
17 human coronaviral infection.
18
- 19 12. Use of interferon as claimed in claims 10
20 or 11 wherein the interferon is interferon
21 alpha or interferon beta.
22
- 23 13. Use of interferon as claimed in claims 10
24 or 11 wherein the interferon is multi-subtype
25 interferon alpha (IFN α), interferon α n1,
26 interferon α n3 or interferon β 1b.
27
- 28 14. Use of interferon as claimed in any one of
29 claims 10 to 13 wherein the interferon is the
30 multi-subtype, human alpha-interferon derived
31 from white blood cells commercially available
32 as Multiferon™.

- 1
- 2 15. Use of interferon as claimed in any one of
- 3 claims 10 to 13 wherein the interferon is
- 4 recombinant.
- 5
- 6 16. Use of interferon as claimed in any one of
- 7 claims 10 to 15 wherein the coronaviral
- 8 infection is a human coronavirus.
- 9
- 10 17. Use of interferon as claimed in any one of
- 11 claims 10 to 16 wherein the coronaviral
- 12 infection is severe acute respiratory syndrome
- 13 (SARS) coronavirus (SARS-HCoV).
- 14
- 15 18. A method of treating human infection with
- 16 a coronavirus, the method including the step of
- 17 administering a therapeutically useful amount
- 18 of an interferon to a subject in need of
- 19 treatment along with a therapeutically useful
- 20 amount of a suitable anti-viral compound.
- 21
- 22 19. A method as claimed in claim 18 wherein
- 23 the coronavirus is severe acute respiratory
- 24 system (SARS) coronavirus (SARS-HCoV).
- 25
- 26 20. A method as claimed in claims 18 or 19
- 27 wherein the anti-viral compound is ribavirin.
- 28
- 29 21. Use of interferon and an anti-viral
- 30 compound in the preparation of a combined
- 31 medicament for the treatment or prevention of
- 32 infection with a coronavirus.

1

2

22. Use of interferon and an anti-viral compound as claimed in claim 21 wherein the coronavirus infection is severe acute respiratory system (SARS) coronavirus (SARS-HCoV).

7

8

9

10

11

23. An assay method for determining the efficacy of a candidate agent in the treatment of a coronaviral infection, the assay method including the steps of;

12

13

14

15

16

17

18

19

20

21

22

23

24

25

24. An assay as claimed in claim 23 wherein the interferon is a multi-subtype interferon.

26

27

28

25. An assay as claimed in claim 24 wherein the multi-subtype interferon is MultiferonTM.

29

30

31

32

26. An assay method for determining the efficacy of a candidate agent in the prevention of a coronaviral infection, wherein the assay method includes the steps of:

1 -incubating cells in the presence of the candidate
2 agent,
3 -adding the coronavirus to the cells, and
4 -determining the degree of protection against the
5 coronaviral infection afforded by the candidate
6 agent.

7

8 27. An assay as claimed in claim 26 wherein
9 the interferon is a multi-subtype interferon.

10

11 28. An assay as claimed in claims 26 or 27
12 wherein the multi-subtype interferon is
13 Multiferon™.

14

15 29. Use of interferon in the manufacture of a
16 medicament for the treatment of a human
17 coronavirus.

18

19 30. Use of interferon as claimed in claim 29
20 wherein the interferon is multi-subtype, human
21 alpha-interferon derived from white blood cells
22 commercially available as Multiferon™.

23

1/4

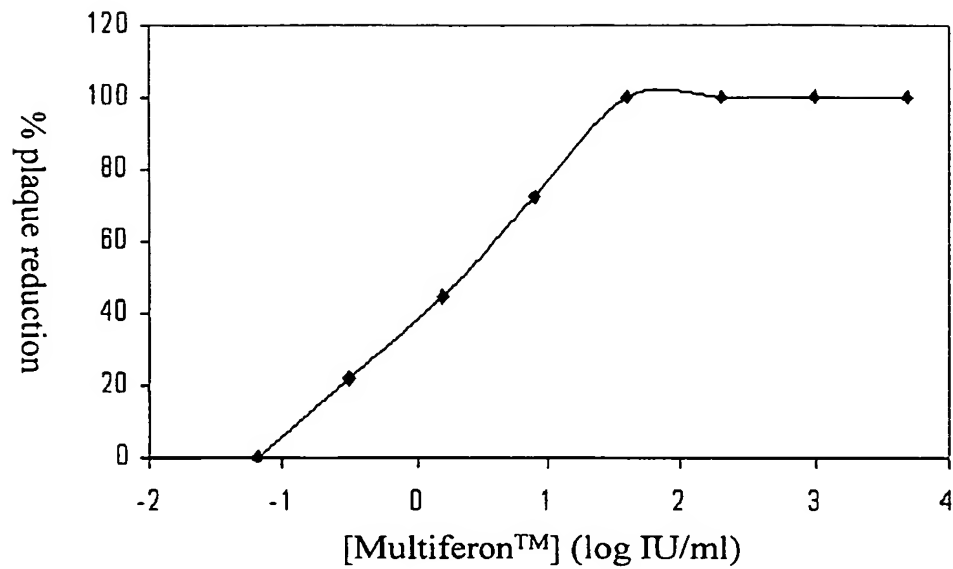


Figure 1: Effect of Multiferon™ against the SARS-HCoV by plaque reduction (Virus concentration is 54 pfu/ml (IU = International Units))

2/4

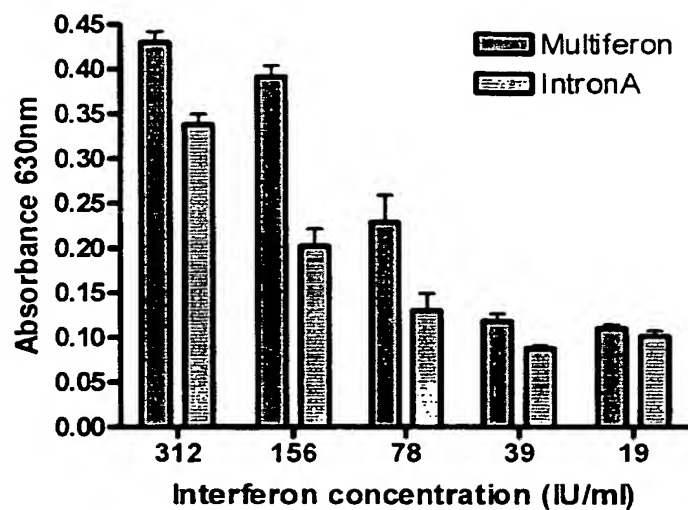


Figure 2: Cytopathic endpoint assay in Vero E6 cells infected with Semliki Forest virus, and treated with a serial titration of Multiferon or Intron A

3/4

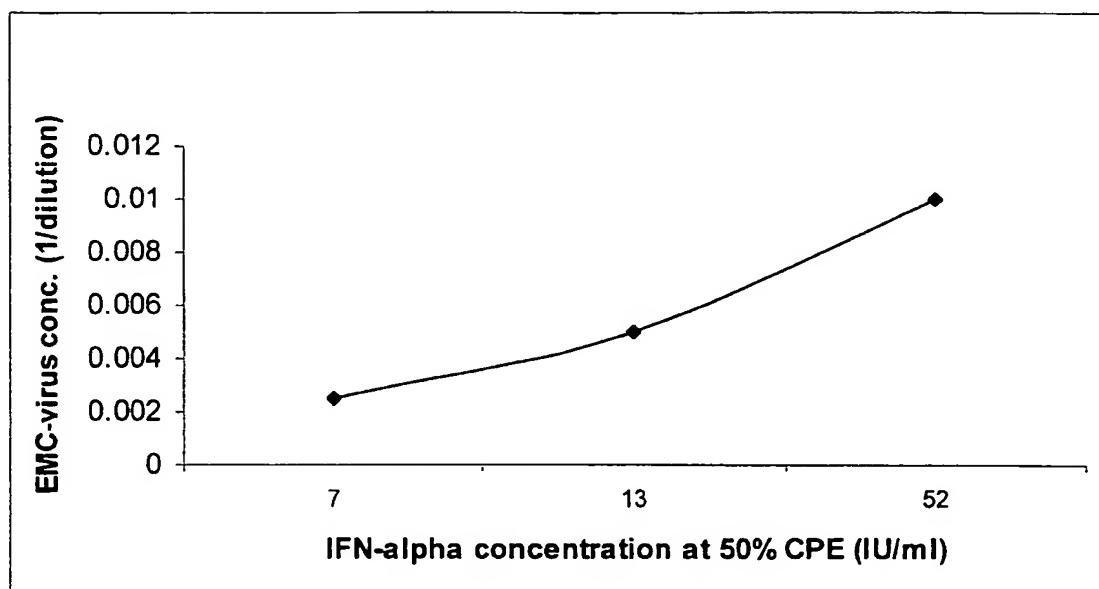


Figure 3: Effect of Multiferon on cytopathogenicity of EMCV on A549 cells. (The Multiferon concentration required to obtain 50 % cytopathic effect (CPE) for human A549 cells challenged with EMC-virus is shown for different concentrations of EMC-virus, presented as 1/dilution).

4/4

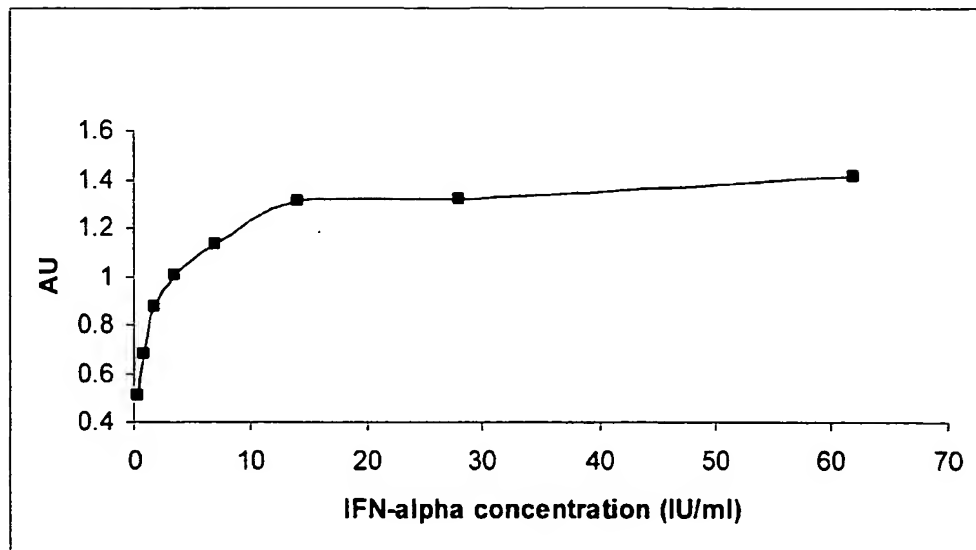


Figure 4: Effect of increasing concentrations of Multiferon™ on survival of A549 cells. Cell survival, measured photometrically at $\text{Abs}_{595\text{nm}}$, using a fixed dilution of EMCV (dilution 1/400), at increasing concentrations of Multiferon. (AU = Absorbance Units)

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/GB2004/002183

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/21 A61K31/7056 A61P31/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBASE, PASCAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CINATL J ET AL: "Treatment of SARS with human interferons" LANCET THE, LANCET LIMITED. LONDON, GB, vol. 362, no. 9380, 26 July 2003 (2003-07-26), pages 293-294, XP004441882 ISSN: 0140-6736 the whole document	1-6, 8-13, 15-17, 23,26,29
P,X	----- DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; June 2003 (2003-06), GAO ZHAN-CHENG ET AL: "'Clinical investigation of outbreak of nosocomial severe acute respiratory syndrome!" XP002294985 Database accession no. NLM12837162 abstract -/--	1,2,5,6, 8-12, 15-22,29

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

6 September 2004

Date of mailing of the international search report

21/09/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo.nl.
Fax: (+31-70) 340-3016

Authorized officer:

Escobar Blasco, P

INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/GB2004/002183

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& ZHONGGUO WEI ZHONG BING JI JIU YI XUE = CHINESE CRITICAL CARE MEDICINE = ZHONGGUO WEIZHONGBING JIJIUYIXUE. JUN 2003, vol. 15, no. 6, June 2003 (2003-06), pages 332-335, ISSN: 1003-0603 -----	
P,X	LAU ARTHUR CHUN-WING ET AL: "Severe acute respiratory syndrome treatment: present status and future strategy." CURRENT OPINION IN INVESTIGATIONAL DRUGS (LONDON, ENGLAND : 2000) AUG 2003, vol. 4, no. 8, August 2003 (2003-08), pages 918-920, XP009035955 ISSN: 1472-4472 page 919, right-hand column, paragraph 4 -----	1,2,6, 8-12, 16-20, 22,29
P,X	ZHAO Z ET AL: "Description and clinical treatment of an early outbreak of severe acute respiratory syndrome (SARS) in Guangzhou, PR China" JOURNAL OF MEDICAL MICROBIOLOGY 01 AUG 2003 UNITED KINGDOM, vol. 52, no. 8, 1 August 2003 (2003-08-01), pages 715-720, XP009035954 ISSN: 0022-2615 treatment group B, C, D page 717 -----	1,2,5,6, 8-12, 15-19, 21,22,29
X	HIGGINS P G ET AL: "INTRANASAL INTERFERON AS PROTECTION AGAINST EXPERIMENTAL RESPIRATORY CORONAVIRUS INFECTION IN VOLUNTEERS" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, US, vol. 24, no. 5, November 1983 (1983-11), pages 713-715, XP009016436 ISSN: 0066-4804 -----	1,2,4-6, 8,10-12, 15,16,29
Y	abstract	3,7,13, 14,30
Y	HUSA P: "What Is the Role of Leucocyte Interferon Alfa in the Treatment of Chronic Hepatitis C in the Time of Pegylated Interferons?" CESKA A SLOVENSKA GASTROENTEROLOGIE A HEPATOLOGIE 2003 CZECH REPUBLIC, vol. 57, no. 5, 2003, pages 189-193, XP009035953 ISSN: 1213-323X abstract -----	3,7,13, 14,30
	----- -/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB2004/002183

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1986, TURNER R B ET AL: "PREVENTION OF EXPERIMENTAL CORONAVIRUS COLDS WITH INTRANASAL ALPHA-2B INTERFERON" XP002294986 Database accession no. PREV198682100287 abstract & JOURNAL OF INFECTIOUS DISEASES, vol. 154, no. 3, 1986, pages 443-447, ISSN: 0022-1899</p> <p>-----</p>	1,2,5,6, 8,10-12, 15,16,29
X	<p>DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; August 2000 (2000-08), MATSUYAMA S ET AL: "Protective effects of murine recombinant interferon-beta administered by intravenous, intramuscular or subcutaneous route on mouse hepatitis virus infection" XP002294987 Database accession no. PREV200000499341 abstract & ANTIVIRAL RESEARCH, vol. 47, no. 2, August 2000 (2000-08), pages 131-137, ISSN: 0166-3542</p> <p>-----</p>	1,2,5,6
X	<p>MYINT S H: "Human coronaviruses: A brief review" REVIEWS IN MEDICAL VIROLOGY 1994 UNITED KINGDOM, vol. 4, no. 1, 1994, pages 35-46, XP009035949 ISSN: 1052-9276 page 43, left-hand column, paragraph 5</p> <p>-----</p>	1,2,8, 10-12, 16,29
A	<p>PEIRIS J S M ET AL: "Coronavirus as a possible cause of severe acute respiratory syndrome." LANCET (NORTH AMERICAN EDITION), vol. 361, no. 9366, 19 April 2003 (2003-04-19), pages 1319-1325, XP002295043 ISSN: 0099-5355 abstract</p> <p>-----</p>	1-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2004/002183

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-20: Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.